The computational prediction of toxicological effects in regulatory contexts

Current use and future potential of (Q)SAR tools

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*La prédiction des propriétés (éco)toxicologiques et physico-chimiques des substances lance de nouveaux défis à la science. Ces défis concernent notamment le monde de la maîtrise des risques, qu'il s'agisse des propriétés toxiques des substances ou encore de leurs propriétés explosives. Dans le cadre de notre dossier relatif au règlement REACH, nous vous proposons ce mois-ci un article dédié aux outils de modélisation et d'étude des relations (quantitatives) structure/activité (en anglais : Quantitative Structure-Activity Relationship ou (Q)SAR), reconnues par le règlement européen REACH sur les produits chimiques comme des outils alternatifs et complémentaires aux outils d'évaluations traditionnels (voir l'*encadré *p. 54 pour la description des méthodes classiques).*

Résumé **La modélisation des effets toxicologiques : utilisation actuelle et potentiel futur des outils (Q)SAR**

Les effets toxicologiques des molécules peuvent être prédits grâce à la modélisation, quantitative ou qualitative, de la relation entre structure chimique et activité biologique. Cette approche de modélisation est connue sous le nom de modélisation (Q)SAR. Depuis le travail pionnier réalisé par Corwin Hansch dans les années 60, le développement et l'utilisation des relations entre structure et activité ont connu un essor croissant ces dernières années, soit pour des applications industrielles, soit pour des applications réglementaires. Cette tendance est de surcroît stimulée par l'implémentation de nouvelles réglementations européennes (REACH, 7e amendement de la Directive européenne sur les cosmétiques) dont le succès passe indéniablement par le déploiement d'une stratégie *in silico* intégrée. Cet article présente de façon synthétique l'utilisation actuelle et le potentiel futur de ces méthodes pour la prédiction des dangers toxicologiques des substances pour l'homme.

Mots-clés **Toxicologie computationnelle, modélisation, (Q)SAR, *in silico*.**

Abstract Quantitative and qualitative models describing the relationship between chemical structure and biological activities can be used to predict toxicological effects of chemicals and they are referred to as (Q)SAR models. Since the pioneering work of Corwin Hansch in the 60's, the development and utilization of structure-activity relationships have become increasingly more important over the past years for industrial and regulatory applications. The implementation of new European chemical safety policies (REACH, 7th amendment of the EU cosmetic Directive) is one of the incentive of this trend whose success is dependent upon the implementation of *in silico* methods among fully integrated strategies. This review briefly summarizes the current utilization and future potential of such modeling approaches for predicting chemical-induced human health hazards.

Keywords **Computational toxicology, (Q)SAR, *in silico*.**

Introduction and historical background

Xenobiotic agents can disturb a biological system in several ways including interactions with endogenous molecular targets (*e.g.* receptors), oxidative stress, interference with normal metabolism, interactions with ion transporters and disruption of calcium homeostasis [1].

It is generally recognized that the molecular structure of chemicals plays a central role in modulating their toxicological activity and such a structure-activity paradigm is of central importance in molecular toxicology. For instance, by analyzing 232 chemicals and their corresponding binding data to the estrogen receptor (ER), Tong *et al.* have shown that 95% (124/131) of all active chemicals matched one or more of the following structural alerts(1): the steroid skeleton, the diethylstilbestrol (DES) skeleton and the phenolic ring [2] (*figure 1*). The identification of such structural motifs is of great toxicological importance because binding to ER is known as one of the potential

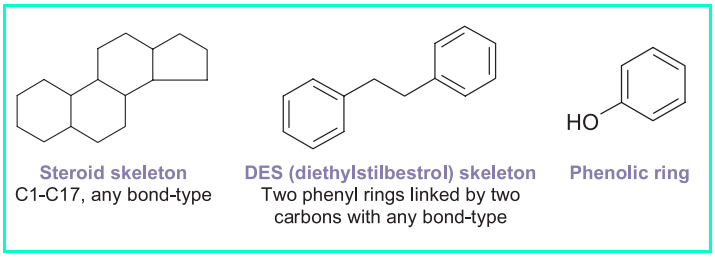

Figure 1 - Three structural alerts observed in most ligands to estrogen receptor (ER) according to [2].
Figure adapted from [2].

mechanisms associated with toxic effects mediated by endocrine disruptors.

Computational models that can be used to analyze structure-activity relationships fall under two broad categories: models based on statistical approaches (artificial-intelligence based systems) and models based on generic toxicological rules derived by experts.

The first category of models relies on mathematical approaches (*e.g.* multivariate regression, discriminant analysis) aimed at describing the relationships between a set of descriptors and toxicological effects. MC4PC, TOPKAT, LAZAR and MDL QSAR fall into this category of models. The analysis of the relationship existing between chemical structure and biological activity can be successfully carried out if chemical structures are characterized by well defined parameters such as structural (atom-based) fragments, physico-chemical, topological, geometrical and quantum mechanical properties. These parameters are commonly referred to as molecular descriptors (*table I*) and their numerical value can in turn be used to define a statistical model describing the correlation between chemical structure and toxicological effects.

On the other hand, the second category of models is based upon the formalization of toxicological knowledge in a set of rules aimed at the recognition of molecular substructures known as structural alerts[1] (also called toxicophores) which are known for having or modulating a toxicological effect. Examples of such expert systems include Derek for Windows, Oncologic and Toxtree. An example of such an approach is given in *figure 2* for Derek and in *figure 3* for Toxtree.

There are also prediction tools that are based on a combination of these two approaches to yield hybrid expert systems (such as TIMES [3]) whose rules are based both on mathematical approaches and expert knowledge.

These basic concepts between structure and toxicological properties are central to qualitative Structure Activity Relationships (SARs) and Quantitative Structure Activity Relationships ((Q)SARs) that, when predicting for toxicological endpoints, are also referred to as *in silico* tools. These tools are complementary to *in vivo* and *in vitro* methods used in toxicology, and their synergistic use is the basis of a number of Integrated Testing Strategy (ITS) which are discussed in a later section of this article.

Computational methods for the prediction of toxicity cover a large variety of toxicological endpoints. Such methods are extensively described in the literature [4] and play an important role in helping to understand the potential mechanism(s) of action of specific classes of chemicals by providing hypothesis than can be validated by *in vitro* or *in vivo* experiments, and thus are helpful in the development of safe entities within the industry.

The first formalization of biological activity as a function of chemical structure is historically attributed to Hansch [5] and his work aimed at modeling the biological activity of compounds that are structurally similar to phenoxyacetic acid, which functions as a plant growth regulator. In this seminal paper, Hansch developed equations which related biological activity to the hydrophobicity and electronic characteristics of benzene by means of the following equation:

$$\log(1/C) = k_1 \mathrm{LogP} - k_2(\mathrm{LogP})^2 + k_3\sigma + k_4$$

where C is the concentration of the compound required to produce a defined level of biological activity, LogP is the

Table I - Some classes of molecular descriptors used in (Q)SAR modeling.

Descriptor typology	Examples
Physico-chemical properties	Molecular weight, LogP, molecular surface area, molar volume, molar refractivity
Topological	Arrangements of atoms, branching and cyclicity
Molecular fields	Computation of the steric and electric potentials surrounding a molecule
Geometrical	Molecular eccentricity, asphericity
Quantum mechanical	Net atomic charges, orbital energies, molecular polarizability

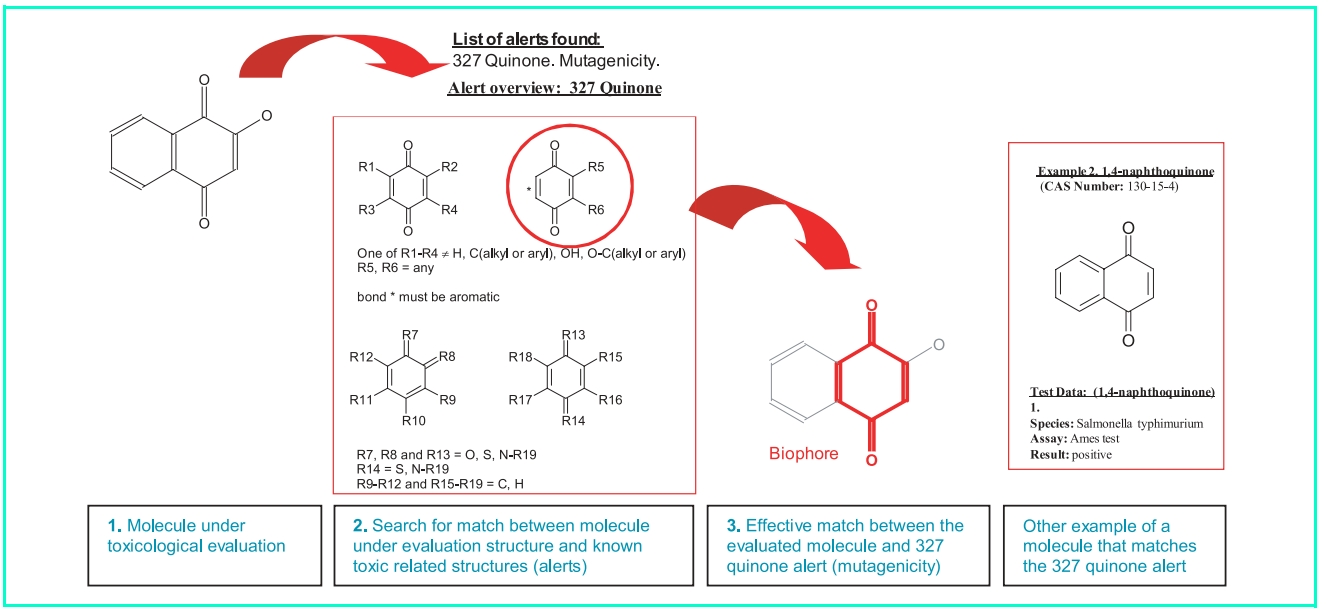

Figure 2 - Example of a Structure Activity Relationships (SAR) obtained by means of the expert system Derek for Windows (DfW) version 11.0.0 where the 327 quinone alert has been identified.
The genotoxicity of quinones is associated with their ability to undergo enzymatic and non-enzymatic redox cycling with their corresponding semiquinone radical. As a result they generate superoxide anion radicals that can be converted to powerfully oxidising hydroxyl radicals that can cause oxidative damage to DNA (source: report from DfW 11.0.0).

Les méthodes classiques d'évaluation des dangers toxicologiques d'une substance chimique

Les études toxicologiques sont la base de toute évaluation des dangers d'une substance chimique. Elles doivent permettre d'identifier les effets résultant d'une exposition, leurs caractéristiques histologiques et d'établir, le cas échéant, des relations dose-réponse. L'évaluation des dangers est la première étape de l'évaluation des risques sanitaires, dont l'objectif est d'assurer l'utilisation d'une substance la plus sûre possible, à la fois pour le travailleur et le grand public.
Depuis 1981, des principes ont été proposés par l'Organisation de Coopération et de Développement Économiques (OCDE) pour que les études réalisées soient reproductibles, comparables et de meilleure qualité, à l'échelle internationale, et ce dans le contexte de l'AMD (Acceptation Mutuelle des Données). Ses principes sont, d'une part, les bonnes pratiques de laboratoire (BPL) et, d'autre part, l'établissement de lignes directrices pour la réalisation d'essais sur les substances chimiques. Des protocoles expérimentaux standardisés ont ainsi été développés pour caractériser au mieux toutes les propriétés d'une substance, notamment ses effets sur la santé. Plusieurs organismes proposent donc désormais des protocoles standardisés tels que l'US EPA(1) (lignes directrices OPPTS(2)), l'ICH(3) ou l'OCDE. Pour sa part, l'Union européenne adopte les lignes directrices établies par l'OCDE.
D'une manière générale, les lignes directrices sont élaborées pour toutes les durées d'exposition possibles (de quelques heures à la vie entière d'un animal), pour l'ensemble des voies d'exposition (orale, respiratoire et cutanée), et ce en fonction de l'effet recherché. Elles sont principalement réalisées chez les rongeurs et les lapins, et dans une moindre mesure chez les singes ou les chiens.

À ce jour, l'OCDE propose 52 lignes directrices, dont certaines sont citées à titre d'illustration dans le texte. Elles permettent d'évaluer :
- ***La toxicité aiguë*** concerne les effets néfastes pouvant résulter d'une exposition unique, ou d'expositions multiples en l'espace de 24 heures à une substance, et permet de déterminer la dose à l'origine d'une mortalité de 50 % (notée DL_{50} pour les voies orale et cutanée, et CL_{50} pour la voie respiratoire).

• ***Essai n° 403*** *: Toxicité aiguë par inhalation.*

- ***La toxicité à doses répétées*** correspond aux effets toxiques généraux se produisant consécutivement à une exposition journalière répétée d'une substance durant une partie (exposition subaiguë ou subchronique) ou la majorité (exposition chronique) de la durée de vie. Les effets systémiques, liés à l'administration de la substance, sont observés au niveau de tous les organes et décrits avec la détermination de la dose sans effet observé (« no observed adverse effect level », NOAEL) ou de la plus faible dose entraînant un effet néfaste pour l'organisme (« lowest observed adverse effect level », LOAEL).

• ***Essai n° 412*** *: Toxicité à doses répétées par inhalation : 28/14 jours.*

- ***Les potentiels irritant/corrosif***, effets locaux pouvant apparaître au niveau du point de contact de la substance avec la peau, l'œil ou un épithélium muqueux tel que le tractus respiratoire. Les substances corrosives sont capables de détruire les tissus vivants avec lesquels elles entrent en contact lors d'une exposition unique. Les substances irritantes sont des substances non corrosives qui, par contact immédiat avec le tissu concerné, peuvent provoquer une inflammation après une exposition unique.

• ***Essai n° 404 :*** *Effet irritant/corrosif aigu sur la peau.*

- ***Les effets sensibilisants*** pour des substances capables de provoquer une réponse allergique chez des individus prédisposés. La sensibilisation peut être cutanée ou respiratoire.

• ***Essai n° 406*** *: Sensibilisation de la peau.*

- ***Le caractère CMR*** : effets cancérigènes (induction de tumeurs, augmentation de leur incidence et/ou de leur caractère malin, ou diminution de la durée de leur apparition), mutagènes (modifications permanentes transmissibles dans la quantité ou la structure du matériel génétique) et reprotoxiques.

• ***Essai n° 416*** *: Étude de toxicité pour la reproduction sur deux générations.*
• ***Essai n° 451*** *: Études de cancérogenèse.*
• ***Essai n° 471*** *: Essai de mutation réverse sur des bactéries.*

Ce type d'études, dont les résultats forment le corps des dossiers réglementaires, est réalisé actuellement au sein de l'Union européenne, aussi bien dans le cadre des biocides, des produits phytopharmaceutiques, que dans les dossiers d'enregistrement requis par le règlement REACH.
Toutefois, pour certains effets critiques particuliers, des méthodes dites « alternatives » sont en cours de validation ou même déjà adoptées afin de limiter le recours à l'expérimentation animale quand cela s'avère possible. Ainsi, des tests *in vitro* ont été développés pour évaluer les potentiels irritant, corrosif, sensibilisant ou mutagène des substances chimiques, à partir par exemple de modèles cellulaires reconstituant la peau humaine pour les effets cutanés. Des approches prédictives de la toxicité par modélisation (voir l'article de E. Mombelli) sont également développées (approches *in silico*).

(1) US EPA : United States Environmental Protection Agency.
(2) OPPTS : Office of Prevention, Pesticides and Toxic Substances.
(3) ICH : International Conference on Harmonisation of technical requirements for the registration of pharmaceuticals for human uses.

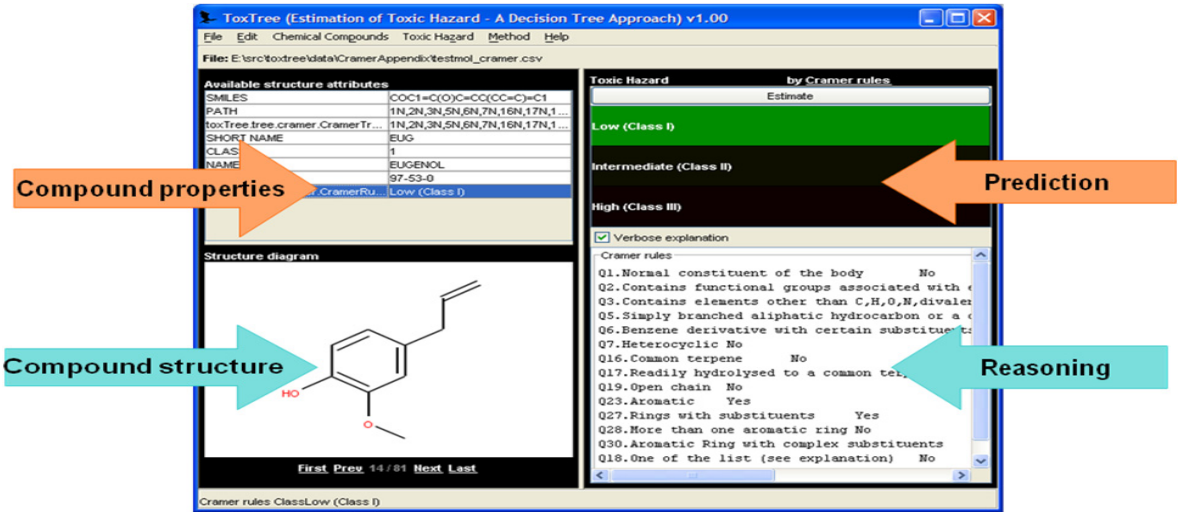

Figure 3 - Predictive output of the software Toxtree which is a flexible, user-friendly application for grouping chemicals and for predicting various types of toxicity based on decision tree approaches.
According to the Cramer decision tree, eugenol is identified as a chemical whose structure does not permit any strong initial presumptions of safety. Toxtree is a software developed by Nina Jeliazkova (Ideaconsult Ltd) on behalf of the Joint Research Centre (JRC). © European Communities (2005, 2007 & 2008). Screenshot kindly provided by Andrew Worth from the Computational Toxicology Group of the JRC.

logarithm of the partition coefficient of the substance between n-octanol and water, σ is the Hammett substituent parameter and k_1-k_4 are constants. The Hammett parameter is a measure of the electron withdrawing or electron donating ability of a substituent and it has been determined by analyzing the dissociation of benzoic acids. This parameter takes into account the effect of ring substituents on ionization. The hydrophobic component (LogP) models the ability of the chemicals to pass through cell membranes. Indeed, Hansch acknowledged that there is an optimal value for LogP: if it is too high the chemical would remain within the membrane, if it is too low the chemical would remain in the aqueous phase without partitioning into the lipids.

The utilization of (Q)SAR models in scientific research and regulatory contexts

(Q)SAR models are extensively used in the pharmaceutical industry in the drug development process (modern medicinal chemistry) with a two-fold objective. In the first place, the selection of promising lead molecules with the desired activity; in the second place, the reduction of expensive drug failures caused by toxicity issues discovered lately in the drug development cycle or in clinical trials [4, 6]. The adoption of (Q)SAR methods is also very important for the replacement, reduction and refinement of the use of animals, the so-called three Rs [7].

(Q)SARs, together with grouping approaches such as chemical categories and read-across[(2)], also play an important role in the evaluation of the toxicity of chemicals (and their safe use) in the new European community regulation REACH (Registration, Evaluation, Authorisation and Restriction of CHemical substances) where they can be used to predict toxicological properties [8]. Indeed, it has been estimated that by applying (Q)SARs and read-across techniques which are currently available, the needs for animal tests could be reduced by up to 70% for some individual endpoints resulting in significant savings in testing costs and use of animals [9]. Several international initiatives are promoting the regulatory use of (Q)SAR methods and more notably the OECD has proposed five principles for their validation in view of their utilization during the regulatory assessment of chemical safety (*table II*) [10]. These principles

Table II - OECD principles for the validation of (Q)SAR models for their use in regulatory assessment of chemical safety.

Principle	Explanation
Defined endpoint	Ideally, (Q)SARs should be developed from homogeneous datasets in which the experimental data have been generated by a single protocol for a given endpoint.
An unambiguous algorithm	The intent is to ensure transparency in the description of the model algorithm so that others can reproduce the model and explain how (Q)SAR estimates are derived.
A defined domain of applicability	(Q)SARs models are inevitably associated with limitations in terms of the types of chemical structures, physico-chemical properties and mechanisms of action for which the models can generate reliable predictions.
Appropriate measures of goodness-of-fit, robustness and predictivity	This principle expresses the need to provide two types of information: a) the internal performance of a model (as represented by goodness-of-fit and robustness), determined by using a training set; and b) the predictivity of a model, determined by using an appropriate external test set (*i.e.* not used during the parametrization of the model).
A mechanistic interpretation, if possible	The intent of this principle is to ensure that there is an assessment of the mechanistic associations between the descriptors used in a model and the toxicological endpoint. A well-substantiated fifth criteria greatly increases the transparency of the model thanks to an explication of causation in terms of molecular interactions.

provide a sound epistemological framework for evaluating regulatory applicability of structure-activity relationships by describing well-defined quality criteria.

The cosmetic industry is currently facing a major challenge with the 7th amendment to the EU cosmetic Directive. The amended directive drew up a list of long term objectives that should be met by 2013. The main points are the following:

• From 2009: ban on the marketing of cosmetic products and ingredients tested on animals for the majority of tests irrespective of availability of a non-animal alternative (among endpoints concerned: genotoxicity, skin and eye irritation and mammalian acute toxicity).

• From 2011: regular meetings with the different stakeholders since some of the challenges of the 7th amendment are of great complexity.

• From 2013: complete sales ban will be in effect for the remaining testing areas (including skin sensitization, reproductive toxicity, repeat dose toxicity, and toxicokinetics).

To sustain innovation, the industry needs alternative methods useful for decision making covering (i) the multiple endpoints currently measured by *in vivo* and *in vitro* assays, (ii) the diversity of mechanisms of action and confounding factors, and (iii) the physico-chemical diversity of "real-life" ingredients. In this context, *in silico* predictive models of toxic effects can be used early in the development/selection process of new chemical entities in order to assist chemists in the priorization of the most promising entities, or at a later stage in the regulatory context for hazard/risk assessment and the constitution of files marketing authorizations. Predictive *in silico* models do not play a decision role as such but are considered in the priorization or decision processes along with other relevant pieces of information.

It is clear that in the frame of such regulatory constraints, capacity-building programs and information sharing strategies in the field of (Q)SAR modeling are needed and are being developed as shown in *figure 4* and *table III*. In order to fulfill these needs, the formerly known European Chemicals Bureau (ECB) was promoting a series of activities including the assessment of already existing (Q)SAR models, the development and harmonization of computational tools and the organization of training courses [11]. The assessment of (Q)SAR models by the ECB and other parties against the OECD principles is driven by the need for an increased and easily accessible information on the scientific quality of existing models.

These efforts are harmonized with the OECD commitment to make available a "(Q)SAR application toolbox"(3) [12] which aims at making toxicological databases, chemical categories, read-across approaches, and (Q)SAR models readily accessible and user-friendly.

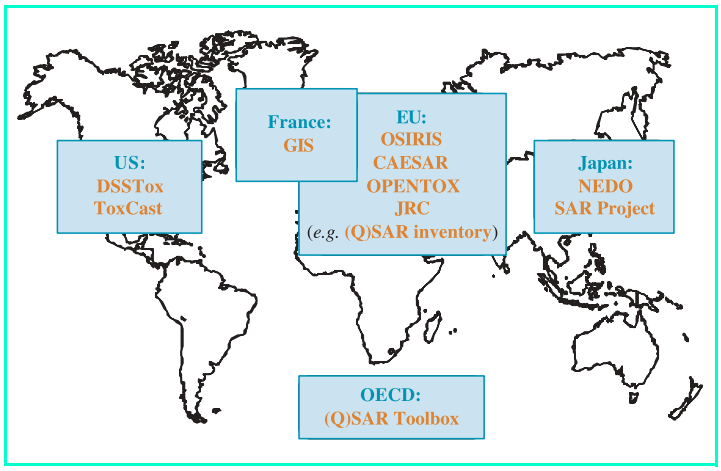

Figure 4 - Examples of national/international initiatives (consortiums, projects, actions) supporting the development/use of computational approaches in predictive toxicology.
Map reproduced with the kind authorization of Professor J.P. Bradu (http://jfbradu.free.fr/cartesvect/index.htm).

Table III - Short description of some of the national/international initiatives (consortiums, projects, actions) supporting the development/use of computational approaches in predictive toxicology.

Program/ Initiative	Description	Reference
DSSTox	Distributed Structure-Searchable Toxicity (DSSTox) is a project aimed at building a public data foundation for structure-activity and predictive toxicology capabilities.	www.epa.gov/NCCT/dsstox/
ToxCast	ToxCast™ is focused on bioactivity profiling and the implementation of computational models to forecast the potential human toxicity of chemicals.	www.epa.gov/ncct/toxcast/
GIS	The GIS (Groupement d'Intérêt Scientifique) aims at facilitating the implementation of alternative methods to animal experimentation.	www.afssaps.fr/Partenariats/Groupement-d-Interet-Scientifique-GIS
OSIRIS	The OSIRIS project aims at developping integrated testing strategies (ITS) fit for REACH that enable to significantly increase the use of non-testing information including (Q)SARs.	www.osiris.ufz.de/
CAESAR	CAESAR is a project which aimed at building (Q)SAR models for the REACH regulation.	www.caesar-project.eu/
OpenTox	The OpenTox project aims at developping a predictive toxicology framework, that provides a unified access to toxicological data and (Q)SAR models.	www.opentox.org/
JRC	The Computational Toxicology Group of the JRC promotes computer-based methods suitable for the regulatory assessment of chemicals.	http://ecb.jrc.ec.europa.eu/qsar/
NEDO	NEDO aims at accelerating the safety evaluations of chemicals and at developing a chemical safety prediction system based on (Q)SARs.	www.nedo.go.jp/english/activities/portal/gaiyou/p00002/p00002.html
OECD (Q)SAR Toolbox	The Toolbox is intended to be used for filling data gaps in (eco)toxicity data using read-across approaches and (Q)SAR models.	www.oecd.org/document/23/0,3343,en_2649_34377_33957015_1_1_1_37465,00.html

(Q)SAR models are also used by the US Environmental Protection Agency (EPA) which, for example, adopts (Q)SARs to estimate the toxicity of chemicals used in industry and discharged into water. The US EPA is coordinating the development of a major data sharing effort for improved structure-activity and predictive toxicology capabilities: the Distributed Structure-Searchable Toxicity (DSSTox) Database Network provides a public forum for publishing downloadable, structure-searchable, standardized chemical structure files associated with toxicity data [13]. Such initiatives provide access to high-quality datasets which can be used for (Q)SAR model development.

The Danish EPA is also very active in the (Q)SAR field and has been working for years in the development and use of computer models for predicting properties of chemical entities. The Danish (Q)SAR database (now maintained by the Danish (Q)SAR Group at the National Food Institute) holds predictions from over 70 models covering a wide range of endpoints (including many human toxicity-related endpoints) for about 166,000 organic chemicals [14].

The Center for Drug Evaluation and Research (CDER) at the US Food and Drug Administration (FDA) is also engaged in a number of initiatives aiming at developing computational toxicology [15].

In the course of toxicological assessments, integration of (Q)SARs with other non-animal methods holds great promises within the framework of an integrated testing strategy whose general flow chart is reported in *figure 5*. The adoption of (Q)SAR predictions within the first tier of an Integrated Testing Strategy (ITS) as an alternative to the bioaccumulation flow-through fish test is proposed by Wolf *et al.* [16]. Similarly, but in a broader framework, the ECETOC Technical Report N° 93 analyzes the utilization of (Q)SAR for the prediction of toxicological effects and physico-chemical properties within a targeted risk assessment [17]. In Hewitt *et al.* [18] the possibility of predicting the placental membrane permeability towards chemicals is proposed as the start of any ITS on reproductive toxicity. A number of EU Framework Programs (*figure 4*) also contribute to broadening scientific knowledge by developing *in silico* tools and/or combining them with other pieces of information available such as *in vitro* tests (*e.g.* the CAESAR, OPENTOX and OSIRIS projects).

(Q)SAR models are widely used for the prediction of toxic hazards such as skin sensitization [19], mutagenicity and carcinogenicity [20]. In order to be predictive of the potential risks associated with chemicals, models have to take into account exposure scenarios. Absorption, distribution, metabolism, excretion (ADME), as well as mixture effects (interactions with other components of a cosmetic formulation for instance), dose, route of administration and frequency of use, are all key parameters to consider when moving from hazard assessment to risk assessment. As such, the process of biotransformation of xenobiotics plays a key role in toxic responses. Therefore computer simulators of tissue metabolism such as those available today (for instance Meteor and TIMES) are needed in order to have a comprehensive knowledge of the metabolic fate of xenobiotics.

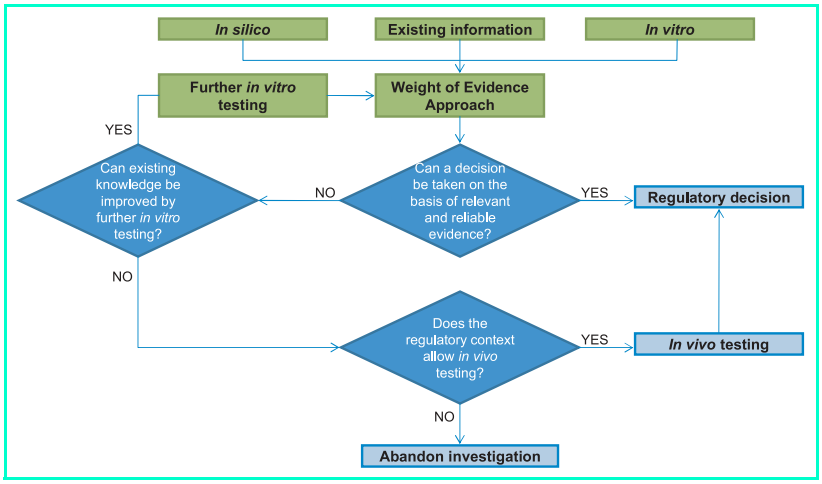

Figure 5 - Example of general flowchart for decision making in a regulatory context. *In silico* predictions can be used at an early stage and their contribution assessed in a weight of evidence approach. Adapted from Grindon *et al.*, ATLA 34, **2006**, p. 407-427.

(Q)SAR modeling: state-of-the-art

Several criteria have to be considered when developing (Q)SAR models among which the quality of data in the training set, the mechanistic rationale for the toxic effect being modelled, the definition of the applicability domain, and the toxic effect itself (see *table II*). All of these criteria will impact the relevance and predictive performance of the model. Some toxicological endpoints represent real challenges to predict, for example reprotoxicity or acute toxicity, considering the multitude of mechanisms involved. Some other endpoints are better understood from a mechanistic point of view – for instance mutagenesis mediated by covalent binding of chemicals to DNA – and therefore have been the primary focus of interest for a large number of models. It is also important to point out that the great majority of available (Q)SAR models predict toxicity for single, pure chemicals and that the development of (Q)SAR approaches for mixture toxicity is still in its infancy.

Availability and quality of training sets

The prediction of toxicological endpoints is often based on models calibrated over toxicological databases whose data content is chemically and mechanistically very diverse. This heterogeneity in dataset composition limits the possibilities of devising a model on the basis of well-defined *a priori* structure-activity hypothesis (*e.g.* a specific interaction with a known receptor). This scenario is in contrast with the area of drug design where chemicals having only minor structural variations with respect to a "lead" chemical are synthesized and then experimentally checked for their biological activity.

Moreover, one must be aware that the structural pattern of chemicals in use in the world is constantly changing and this means that the available training sets for (Q)SAR parameterization are never up-to-date because they do not include toxicological information on newly synthesized structural motifs [21].

From a statistical point of view, toxicological databases tend also to be populated mainly by information on toxic chemicals because costly scientific research is prioritized and targeted towards substances that are reasonably believed to be hazardous. This statistical bias is then propagated throughout semi-empirical models (such as (Q)SARs) that will therefore tend to over-predict toxicity. The development of commercial models that are transparent in terms of data

sources is usually based upon the same training sets containing data from the public domain. In the course of the development of *in silico* models, an ideal situation is often reached when both toxicologists (or biologists) who know very well the data and the model developer can closely interact, thus preventing a misuse or misinterpretation of the data.

Applicability domain of models

Predictive models can provide a valuable support for the screening and categorization of chemicals in addition to further understanding of mechanistic rationale. However applicability domains often have to be adapted to the needs of the end-user. Indeed it has been observed that models performing well for a given class of chemicals (*e.g.* drugs, industry chemicals, food additives) do not necessarily perform well for other classes since chemical spaces of interest often differ from the one covered in the models training sets [22].

Local versus global models

Global (Q)SARs for non-congeneric sets of chemicals generally provide a first level of information. Such models are based on large and diverse chemical libraries and thus have a large scope of application, but lower prediction accuracy is to be expected [7]. Local (Q)SARs dedicated to congeneric chemicals – *e.g.* aldehydes as ingredients of fragrances – provide additional valuable knowledge to better understand potential mechanisms of action. As such, local (Q)SARs are often developed to answer a question specific of a chemical class of interest. Experimental data generated according to a given protocol for a given endpoint can be used to refine existing global models and to build local models if necessary.

A number of global (Q)SAR models are commercially available. Building an *in silico* strategy based on more than one such model is interesting in view of the differences in the models (SAR *versus* (Q)SAR, expert-knowledge based systems *versus* artificial intelligence-based systems) and applicability domains (chemical space and toxicological endpoint coverage). The predictive performance of commercial models has to be assessed on a regular basis, given the chemical diversity and reactivity of new chemical entities, and regular updates in the software versions.

Predictive performances of models

As a general remark, it should be noted that data used to calibrate structure-activity relationships are characterized by an unavoidable experimental uncertainty and the resulting (Q)SAR model cannot be expected to produce results that are more precise than the degree of accuracy with which toxicological effects can be experimentally described. The use of external test sets is critical to studies aiming at the evaluation of model performances but is only possible when enough good quality data is available.

From a toxicological viewpoint, it can also be added that the predictive performance of (Q)SAR models can be enhanced if they are based on the modeling of a well-understood mechanism. Simon-Hettich *et al.* [23] highlighted the epistemological advantages of dissecting complex toxicological phenomena into several more mechanistically understood endpoints. Given the complexity and diversity of mechanisms involved in toxic responses, *in silico* predictions should therefore be critically evaluated in a context-dependent environment, on a case-by-case basis, along with human expertise.

Perspectives for (Q)SAR modeling

The increasing availability of data mining tools and curated structure-toxicity databases combined to regulatory requirements for the generation of reliable toxicological information are favorable to the future development of effective (Q)SAR models.

In this context, the US EPA has begun a new research effort, the ToxCast™ Program for Prioritizing Toxicity Testing of Environmental Chemicals, to develop the ability to forecast toxicity based on bioactivity profiling (using both high-throughput screening and toxicogenomic technologies). Within ToxCast™, data will be generated on an environmental chemical library using numerous types of assays evaluating a broad spectrum of bioactivities. These data will be relationally linked within the ToxCast™ database to chemical structures, physico-chemical, toxicological, and computer-simulated information, and a strategy including structure-activity relationships is under development to predict toxicity profiles based on the entire dataset. ToxCast™ data and predictions will then be applied to the process of prioritizing environmental chemicals.

Moreover, the impact of the worldwide efforts in toxicogenomics research will also help in providing insight into many aspects of unknown toxicological mechanisms. The unraveling of this “omics” information will in turn prompt the generation of mechanistic-driven (Q)SAR modeling.

From a physico-chemical point of view, the ordinary desktop computers can now easily compute quantum descriptors and in the near future, thanks to the on-going evolution of microprocessors, it will be possible to compute these descriptors for even larger molecules. This progress will allow routine analysis of the intermolecular forces that determine the biological activity of macromolecules.

The integration of Physiologically Based Toxicokinetic/Toxicodynamic[4] (PBPK/PD) and (Q)SAR modeling also represents an interesting and promising field of research. In this integrated scheme, (Q)SAR models provide interpolation for toxicological responses and pharmacokinetic parameters. Indeed, this synergy between the two modeling approaches can greatly reduce the need for animal testing while optimizing in cost-efficient ways toxicological resources.

We should see in the coming years the development of more and more useful and efficient *in silico* models to predict human health hazards although there is still some way to go. Some key milestones include the full coverage of toxicological endpoints (one major current limit being the shared access to high-quality databases) and the coverage of many diverse chemical entities (including natural extracts, polymers, silicones, etc). These milestones as well as the successful implementation of (Q)SAR models within integrated testing strategies are dependent upon an R&D commitment between partners from industry, academy and regulatory.

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Notes and references

(1) *Structural alert*, also called *toxicophores*: molecular substructures which are known for having or modulating a toxicological effect. An example is given in *figure 2*.
(2) *Read-across*: methodology based on the principle of toxicological analogy among chemicals whose physico-chemical, toxicological and ecotoxicological properties are likely to be similar or follow a regular pattern as a result of structural similarity.
(3) A guidance document has recently been issued: www.oecd.org/document/23/0,3343,en_2649_34377_33957015_1_1_1_37465,00.html
(4) Computational models that describe the processes of uptake, distribution, metabolism, and excretion of a xenobiotic in order to study its effects in the body over time.

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